Chapter 2

Platelet Serotonin Levels in Pervasive Developmental Disorders and Mental Retardation: Diagnostic Group Differences, Within-Group Distribution, and Behavioral Correlates

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Abstract

Objective: To investigate group differences, the within-group distributions, and the clinical correlates of platelet serotonin (5-HT) levels in pervasive developmental disorders (PDD). Method: Platelet 5-HT levels were measured in Dutch children and young adults, recruited from 2001 through 2003, with PDD (autism, Asperger's, and PDD-not otherwise specified [PDD-NOS]; n = 81) or with mental retardation (MR; n = 54) but without PDD, and in normal controls (n = 60). The distribution of platelet 5-HT levels was assessed using mixture-modeling analyses. Relationships between platelet 5-HT levels and a full range of demographic, clinical, and behavioral variables were examined. Results: Group mean (± SD) platelet 5-HT levels (nmol/10^9 platelets) were significantly higher in the autistic (4.51 ± 1.61, n = 33) and PDD-NOS (4.90 ± 1.54, n = 43) groups compared to the MR (3.48 ± 1.33, n = 54) or the normal control (3.58 ± 1.08, n = 60) groups ($F_{4,190}$ = 9.35, p < .001). Platelet 5-HT values in the combined PDD group showed a bimodal distribution, and an empirical cutpoint for hyperserotonemia was determined. None of the behavioral variables examined was significantly associated with platelet 5-HT levels. Conclusions: The platelet hyperserotonemia of autism was replicated in Dutch subjects. Platelet 5-HT levels were also increased in PDD-NOS, while no elevation was seen in MR. Platelet 5-HT levels appeared to be bimodally distributed in the PDD group, with an apparent hyperserotonemic subgroup.

Introduction

Platelet hyperserotonemia is a longstanding finding in autism (Schain and Freedman, 1961). Although many studies have been conducted attempting to clarify this phenomenon, no definite conclusions can be drawn regarding the mechanism of the elevated group mean serotonin (5-hydroxytryptamine [5-HT]) levels seen in autism (Anderson et al., 1990; Cook and Leventhal, 1996; Anderson, 2002). A number of investigations have attempted to characterize the group differences in platelet 5-HT levels and sought to identify clinical correlates in the autistic group. A range of demographic, descriptive, and behavioral variables have been evaluated for their relationship with platelet 5-HT levels, including age, sex, ethnicity, family loading, medication, and platelet count and size (Ritvo et al., 1970, 1971; Kuperman et al., 1985; Anderson et al., 1987b; Geller et al., 1988; Abramson et al., 1989; Minderaa et al., 1989; Leventhal et al., 1990; Cook et al., 1990; Piven et al., 1991; Cook et al., 1993; Cuccaro et al., 1993; Anderson et al., 1996; Leboyer et al., 1999). A smaller number of studies have examined the relationship of platelet 5-HT levels to intelligence and mental retardation, and specific aspects of cognitive functioning (Campbell et al., 1975; Cook et al., 1993; Cuccaro et al., 1993; Kuperman et al., 1987; McBride et al., 1998). Most of the studies have addressed only one or two of the variables of interest and have studied limited numbers of subjects.

In the present study we investigated the group differences and the clinical and behavioral correlates of platelet 5-HT levels in relatively large groups of Caucasian subjects with autism, Asperger's syndrome, or pervasive developmental disorder-not otherwise specified (PDD-NOS), and in ethnically matched nonautistic mentally retarded and normal controls. Given the prior reports, we hypothesized that 5-HT levels would be elevated in the PDD groups and unchanged in mental retardation (MR). We specifically aimed to enhance the utility of the platelet 5-HT phenotype by attempting to define biochemical subgroups within the PDD groups and by exploratory analysis of the relation between platelet 5-HT levels and behavioral expression.

Method

Subjects

Children and young adults with pervasive developmental disorders (PDD) or with MR in the absence of a PDD were recruited through an epidemiological survey

being carried out in the north of the Netherlands, and through an autism outpatient clinic affiliated with the Child and Adolescent Psychiatry Center of Groningen. The age range for subjects to be included in the study was 5 to 20 years, corresponding to the school-age range used as inclusion criterion in the epidemiological survey. Subjects were excluded from the study if they had a known genetic condition or severe peri- or prenatal problems. None of the subjects was physically ill or had a history or signs or symptoms of inborn errors of metabolism or chromosomal syndromes, including fragile X syndrome. Thirty of the 81 PDD subjects were receiving medication: 8 were receiving neuroleptics (either the atypical neuroleptic risperidone or pimpamperone, a 'typical' neuroleptic used to treat aggression in MR individuals and in patients with PDD), 7 were receiving methylphenidate, 5 were receiving clonidine, and 10 were receiving antiepileptic drugs. Non-MR normal control subjects were recruited from children seen during routine visits to a pediatric nephrology outpatient clinic of the University Medical Center Groningen. Potential normal control subjects who attended special education schools or who had ever used child psychiatric services were excluded. All subjects in the study were of Dutch descent. Demographic and clinical data for the included subjects are given in Table 2.1. The study was approved by the Medical Ethical Committee of the University Medical Center Groningen, and written informed consent was obtained.

Clinical Assessment

All developmentally impaired subjects were extensively evaluated. PDD subjects were initially evaluated using the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994) and the Autism Diagnostic Observation Schedule-G (ADOS-G; Lord et al., 2000) administered by trained examiners. They were then diagnosed by experienced clinicians using DSM-IV-TR criteria (American Psychiatric Association, 2000). The clinicians were blinded to the ADI-R and ADOS-G outcome. A final diagnosis was established by combining DSM-IV-TR, ADI-R, and ADOS-G information for a best-estimate diagnosis.

To ensure the absence of a PDD in all MR comparison group subjects, the Autism Behavior Checklist (ABC; Krug et al., 1980) was filled out by the parents and the Scale for Pervasive Developmental Disorders in the Mentally Retarded (PDDMRS; Kraijer, 1999) was filled out by teachers and school psychologists. To be

included in the MR comparison group, subjects had to score below the cutoff for PDD on both instruments.

Table 2.1: Demographic and Clinical Characteristics of Subjects

Group	Autism	Asperger	PDD-NOS	MR	Normal Control	Statistic$_{df}$; p
n	33	5	43	54	60	
Age, yr ± SD	11.7 ± 4.0	13.2 ± 3.3	13.1 ± 4.2	13.0 ± 3.3	11.5 ± 3.9	$F_{4,190}$=1.74;.14
Gender (male:female)	29:4	4:1	37:6	43:11	29:31	X^2_4=27.2;<.001
Level of functioning (n)						
Profound/severe MR	10	—	8	2	—	
Moderate MR	10	—	6	15	—	
Mild MR	8	—	17	37	—	
'High-functioning' (>70 IQ)	5	5	12	—	—	X^2_9=56.8;<.001
Vineland scores: total age eq., mo ± SD	44.3 ± 26.8	86.3 ± 3.5	65.1 ± 32.4	70.3 ± 28.2	—	$F_{3,121}$=7.96;<.001
Behavioral ratings						
ADI social	22.1	20.0	15.7	—	—	$F_{2,80}$=12.1;<.001
ADI communication	14.4	15.0	12.1	—	—	$F_{2,80}$=2.92;.06
ADI stereotypies	6.2	5.2	4.8	—	—	$F_{2,80}$=2.50;.09
ADI total	46.8	43.6	36.2	—	—	$F_{2,80}$=8.98;<.001
CBCL internal	59.1	64.6	56.4	53.3	—	$F_{3,132}$=3.42;<.05
CBCL external	52.3	61.2	55.0	52.1	—	$F_{3,132}$=1.30;.28
CBCL total	60.8	65.8	60.4	55.5	—	$F_{3,132}$=3.39;<.05
ABC total	62.8	67.2	42.6	18.5	—	$F_{3,132}$=33.1;<.001
CY-BOCS total	8.6	4.2	5.8	1.8	—	$F_{3,132}$=14.1;<.001
CSBQ total	38.2	42.8	33.6	20.1	—	$F_{3,132}$=11.7;<.001

Note: PDD-NOS = pervasive developmental disorder-not otherwise specified; MR = mental retardation; ADI = Autism Diagnostic Interview; CBCL = Child Behavior Checklist; ABC = Autism Behavior Checklist; CY-BOCS = Children's Yale-Brown Obsessive Compulsive Scale; CSBQ = Children's Social Behavior Questionnaire.

Intellectual functioning was tested in the majority of subjects in the PDD and MR groups. However, because test data were not available for all subjects, subjects were assigned to one of four levels of intellectual functioning: profound/severe MR (total IQ < 36), moderate MR (IQ 36–50), mild MR (IQ 51–70), and 'high' functioning (IQ > 70). In most cases (115/135), this classification was based on information from standardized intelligence tests or developmental tests (Wechsler Intelligence Scale for Children- Revised [WISC-R]; Vander Steene et al., 1986), Wechsler Preschool and Primary Scale for Intelligence [WPPSI]; Vander Steene and Bos, 1997), nonverbal intelligence tests (Snijders-Oomen Niet-verbale intelligentie test; Snijders

et al., 1996) or a Dutch modification of the Bayley scales of Infant Development (Van der Meulen and Smrkovsky, 1983). In 20 of the cases, subjects were assigned to one of these categories by a psychologist (AdB) based on their Vineland Adaptive Behavior Scales Scores (Sparrow et al., 1984), and a clinical review of functioning. No data on intellectual functioning were available for the normal control group.

Information from the parent interview ADI-R was used to assess the severity of social impairment, communication impairment, and stereotypical behaviors. The ADI-R uses a semistructured interview format; for most items, parents are asked about the period between age 4 to 5 years and the present. We chose to use the current scores of the ADI-R to assess relationships between behavior and 5-HT levels ('direct eye gaze' was omitted since this item is only scored before age 6).

For the assessment of speech and language development, three items from the ADI-R were used: 'age at first single words', 'age at first phrases' and 'overall level of language'. The first two items were dichotomized into not delayed and delayed, corresponding to words before or after 24 months and phrases before or after 36 months, respectively.

The Dutch version of the Child Behavior Checklist (CBCL; Achenbach and Edelbrock, 1981; Verhulst et al., 1990) was filled out by parents to explore the presence and extent of other problem behaviors. The Dutch Children's Social Behavior Questionnaire (CSBQ; Luteijn et al., 2000) further evaluates the social behavior of children and has six empirically derived scales: Acting Out, Social Contact Problems, Social Orientation Problems, Social Insight Problems, Stereotypical, and Anxious/Rigid scale. To assess compulsive behavior and stereotypes, the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al., 1997) was administered using parental informants. The ABC (Krug et al., 1980) subscores and total score were used to assess the severity of autistic behaviors in the PDD and MR groups.

Laboratory Measures

Blood samples were collected in 10 mL Vacutainer tubes (Becton-Dickinson, Meylan Cedex, France) containing 0.12 mL (0.34 mol/L) EDTA solution. Platelet-rich plasma was prepared from EDTA blood within 1 hour after sampling by centrifuging for 30 min at 120 × g and 4°C, and a platelet count was obtained. Automated analysis of plasma indoles was performed in batch. Concentrations of 5-HT were

expressed as nmol/10^9 platelets; 5-HT was determined in a quality control sample with within-series and between-series coefficients of variation of 2.8% and 5.4%, respectively (Kema et al., 2001).

Statistical Analyses

The statistical analyses were performed using the SPSS software package (SPSS Inc., 1999) and the Analyse-It software for Microsoft Excel (Analyse-It Software Ltd., 2002). Possible diagnostic group differences in demographic variables and behavioral ratings were analyzed using analysis of variance (ANOVA) or X_2 test where applicable. Relationships between platelet 5-HT levels and demographic variables were evaluated with ANOVAs and Students t-tests. Given some nonnormal subgroup distributions, both ANOVA and the Kruskal-Wallis (KW) test were used to compare platelet 5-HT levels, platelet count, and mean platelet volume between diagnostic groups. Post hoc group comparisons were performed using the Tukey honestly significant difference test and the Mann-Whitney U test. To assess the effect of puberty in the absence of Tanner stage information, prepubertal (subjects 10 and younger) and postpubertal (14 and older) groups were formed on the basis of age.

Normality of the distribution of 5-HT levels in the diagnostic groups and subgroups was tested using the Anderson/Darling goodness-of-fit test (Anderson and Darling, 1952; Lewis, 1961). We used the MIXMOD (MIXture MODeling) program to assess whether the sample consisted of one or a mixture of two or more gaussian distributions (Biernacki et al., 2001). This mixture-model approach to commingling calculates approximate Bayes factors or the posterior odds for one model against the other, assuming neither is favored a priori. The smaller the log Bayes factor, termed the bayesian information criterion, the stronger the evidence for the model (Fraley and Raferty, 1998; Schwartz, 1978).

Relations between the behavioral measures and platelet 5-HT levels were evaluated using Spearman rank correlations. Nonparametric tests were used, since not all behavioral measures showed normal distributions. When significant correlations were found, further analyses were performed using linear and logistic regression analysis, with the platelet 5-HT level as the dependent variable and the behavioral measures as independent variables.

The α-value was set at $p = .05$ for all analyses. To avoid errors of the second type in the exploratory analyses, no correction for multiple comparisons was made.

Effects of age, gender, season, and medication were tested due to prior reports indicating these variables might have some effect on platelet 5-HT levels.

Results

5-HT Level and Age, Gender, Level of Functioning, Use of Medication, and Season

The variables age, gender, level of functioning, use of medication, and season of blood draw were included in an univariate analysis of variance (ANOVA) to test their effect on platelet 5-HT levels in all subjects. The effects of age, gender, level of functioning, use of medication, and season were not significant ($F_{1,194}$ = 0.03, p = .87; $F_{1,194}$ = 2.12, p = .15; $F_{3,194}$ = 0.35, p = .70; $F_{3,194}$ = 1.19, p = .31; and $F_{3,194}$ = 2.24, p = .090, respectively). No significant interaction effects were observed between any of the variables.

Table 2.2: Platelet 5-HT Levels, Platelet Count, and Mean Platelet Volume in PDD, MR, and Normal Control Groups

Subject Group	***n***	***5-HT[a,b] (nmol/10⁹ plts)***	***Platelet Count (10⁹/mL)***	***Mean Platelet Volume (fL)***
Autism	33	4.51 ± 1.61	0.428 ± 0.130	8.17 ± 0.95
Asperger	5	4.00 ± 1.37	0.463 ± 0.158	7.42 ± 0.14
PDD-NOS	43	4.90 ± 1.54	0.408 ± 0.118	8.27 ± 1.13
Combined PDD[c]	81	4.69 ± 1.56	0.429 ± 0.131	8.13 ± 0.97
MR	54	3.48 ± 1.33	0.400 ± 0.119	7.84 ± 1.09
Normal control	60	3.58 ± 1.08	0.423 ± 0.151	8.17 ± 1.01
Kruskal-Wallis		X^2_4 = 30.1	X^2_4 = 2.85	X^2_4 = 7.16
		p < .001	p = .58	p = .13
ANOVA		$F_{4,190}$ = 9.35	$F_{4,190}$ = 1.02	$F_{4,190}$ = 1.76
		p < .001	p = .40	p = .14

Note: 5-HT = serotonin; PDD-NOS = pervasive developmental disorder-not otherwise specified; MR = mental retardation; ANOVA = analysis of variance; HSD = honestly significant difference test; plts = platelets.
a. Post hoc M-W U: autism vs. MR and normal p<.005, p=.01, respectively. PDD-NOS vs. MR and normal, both p<.001.
b. Post hoc Tukey HSD: autism vs. MR and normal p=.005, p=.01, respectively. PDD-NOS vs. MR and normal, both p<.001.
c. ANOVA (5-HT), combined PDD vs. MR and normal, F2,194 = 17.2, p<.001, post hoc Tukey HSD, both p<.001. Combined PDD = autism, Asperger, and PDD-NOS.

5-HT Values in Diagnostic Groups

Group mean platelet 5-HT values are given in Table 2.2. An ANOVA confirmed a robust and statistically significant effect of diagnosis. Post hoc tests showed that platelet 5-HT levels were significantly elevated in the autistic group versus the MR and normal control groups and in the PDD-NOS group versus the MR and normal control groups. The group means seen for platelet 5-HT levels in the autism and PDD-NOS groups were 28.7% and 36.9% greater, respectively, than the mean

observed in the normal control group. When individuals with autism, Asperger's, or PDDNOS were combined in one group, an ANOVA also revealed a significant elevation of 5-HT in the combined PDD group. There were no significant differences in platelet 5-HT between the Asperger's, MR, and normal control groups (all three pairwise p values > .05). Platelet count and mean platelet volume did not differ significantly between any of the groups. Medication status did not influence platelet 5-HT levels in the autism group ($F_{3,32}$ = 0.55, p = .65) or the PDDNOS group ($F_{3,42}$ = 0.45, p = .72) or in the combined PDD group ($F_{3,80}$ = 0.40, p = .76).

5-HT Values and Pubertal Status

Given prior reports of pubertal effects on platelet 5-HT levels, the effect of puberty and the interaction effect of puberty and diagnosis were examined in detail in the combined PDD (autism, Asperger's, and PDDNOS) and combined control (normal and MR) groups. When pubertal status and diagnosis were included as predictor variables in an ANOVA of all subjects (post-, peri- and prepubertal, n = 195), the effect of diagnosis was significant ($F_{4,195}$ = 8.89, p < .001), while the effect of puberty and the interaction effect were nonsignificant ($F_{2,195}$ = 0.13, p = .88 and $F_{4,195}$ = 0.95, p = .50, respectively). Diagnostic group comparison in the prepubertal subgroup revealed a significant difference for platelet 5-HT levels between PDD (n = 32) and control (n = 38) subjects (4.81 ± 1.43 versus 3.58 ± 1.24 nmol/10^9 platelets, t_{69} = 4.0, p = .001). A similar significant increase in the PDD group (n = 39) compared to the control group (n = 40) was also observed in the postpubertal group (4.52 ± 1.54 versus 3.61 ± 1.12 nmol/10^9 platelets, t_{70} = 3.0, p = .004). No significant diagnostic group differences occurred in platelet count or mean platelet volume in either pubertal subgroup.

No significant effects of age on platelet 5-HT levels were observed in either the prepubertal ($F_{6,47}$ = 0.45, p = .84) or postpubertal ($F_{8,63}$ = 1.25, p = .29) groups; the interactions between age and diagnosis were also not significant in either group ($F_{12,47}$ = 0.92, p = .53 and $F_{23,65}$ = 1.49, p = .14, respectively).

Distribution of 5-HT Values

Distribution histograms for 5-HT values in normal control, MR, and combined PDD groups are shown in Figure 2.1. The distribution in the PDD group was clearly nonnormal and apparently bimodal on visual inspection. Anderson-Darling tests of

normality indicated that 5-HT values in the normal control group were normally distributed (A^2_{60} = 0.441, p = .29), but that the distributions in the MR and combined PDD groups were nonnormal (A^2_{54} = 1.55, p < 0.001 and A^2_{81} = 0.78, p = .046, respectively).

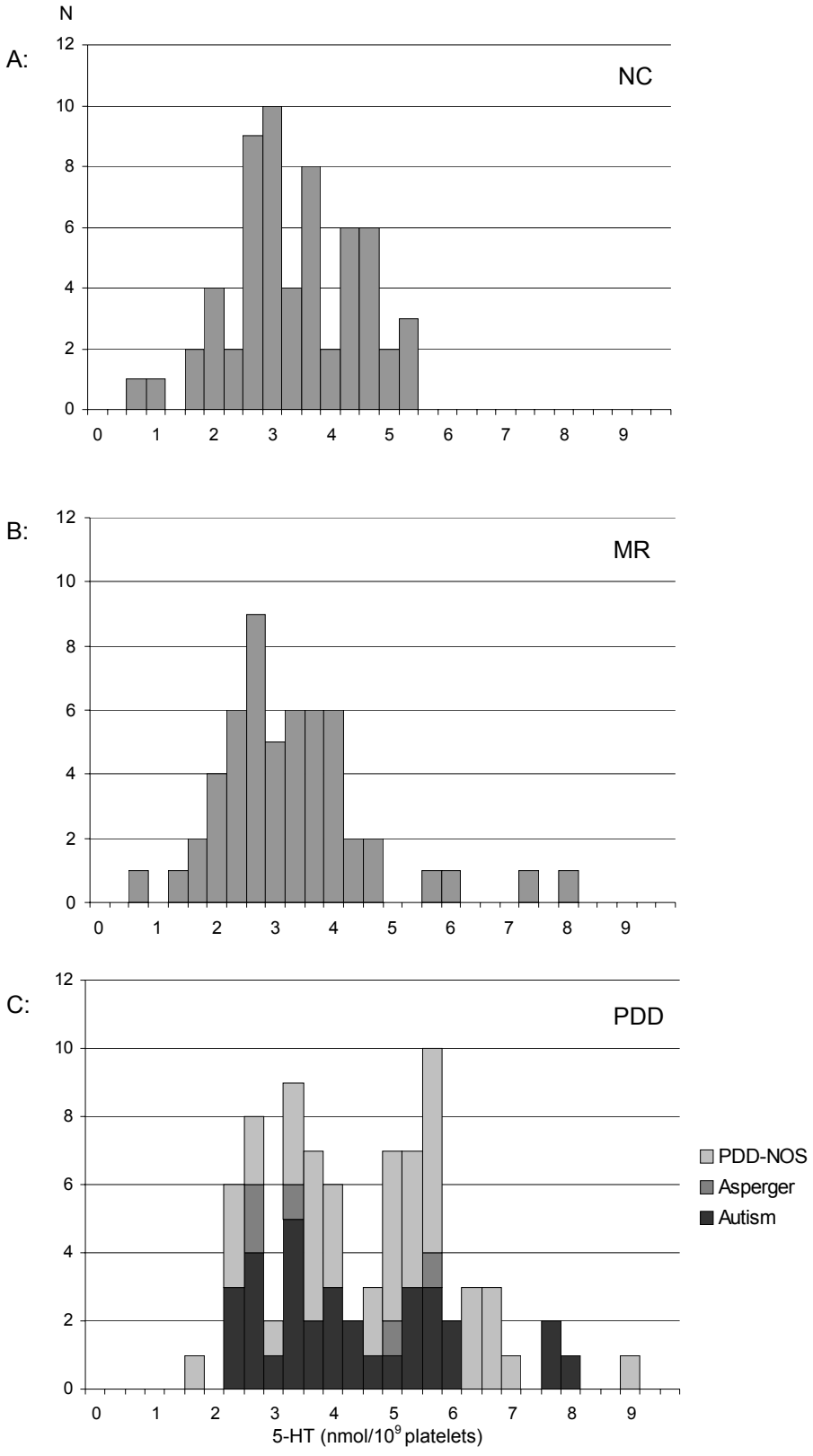

Figure 2.1: Histograms showing the distribution of platelet serotonin (5-HT) levels in the normal control group, n=60 (A), the mentally retarded (MR) group, n=54 (B) and the combined pervasive developmental disorder (PDD) group (autism, Asperger's, and pervasive developmental disorder-not otherwise specified [PDD-NOS]), n=81 (C).

The distributions of platelet 5-HT values in the autism and PDD-NOS subgroups also appeared bimodal by visual inspection, but tests of nonnormality were hindered by reduced sample size (A^2_{33} = 0.73, p = .057 and A^2_{43} = 0.29, p = .60, respectively). The lower mode in the combined PDD group appeared similar in mode, median, and variance to the group distributions observed for the normal control and MR groups. Specifically, if the combined PDD group was dichotomized into normo- and hyperserotonemic subgroups using a valley cutoff value of 4.55 nmol/10^9 platelets, the lower and upper modes had means and SDs of 3.39 ± 0.67 and 6.02 ± 0.98 nmol/10^9 platelets, respectively (t_{79} = 14.1, p < .001). Although a greater proportion of the individuals in the PDD-NOS group were defined as hyperserotonemic using this cutoff value (25/43 [58%] versus 12/33 [36%] in the autistic group), the difference obtained only trend level significance (X^2_1 = 3.54, p = .060). While the mean platelet 5-HT concentration in the small Asperger's group was not elevated, the five observed values (2.7, 2.9, 3.5, 5.2, 5.7 nmol/10^9 platelets) were not inconsistent with the bimodal distribution seen for the combined PDD group.

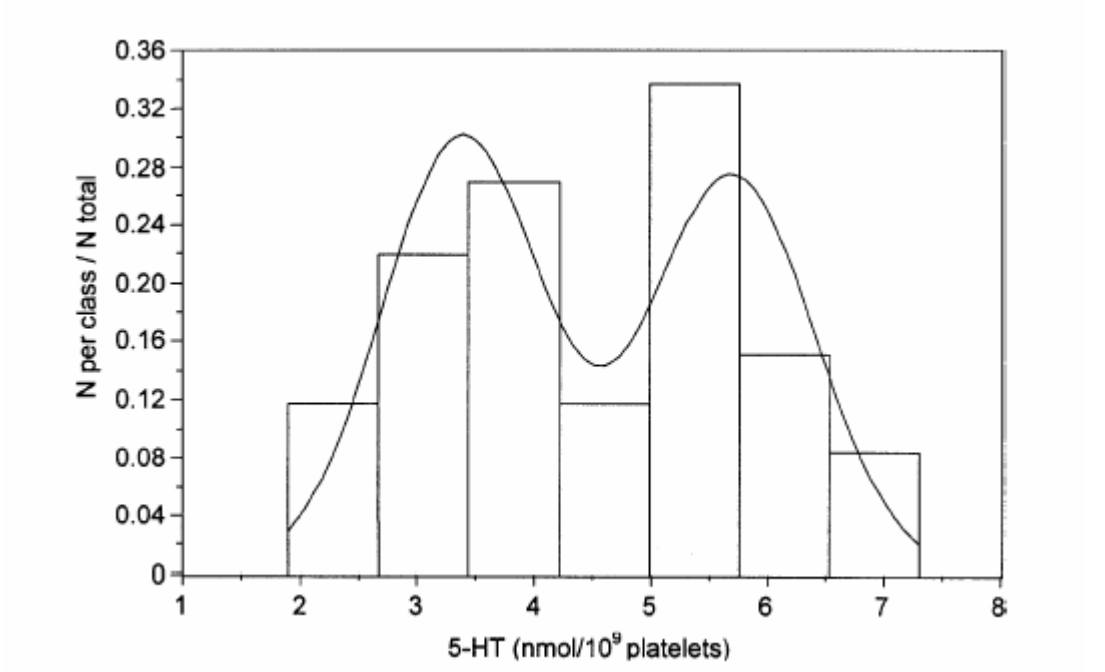

Figure 2.2: The two-component distribution of platelet serotonin (5-HT) (nmol/10^9 platelets) values in the pervasive developmental disorder (PDD) group as determined by commingling analyses with MIXMOD. The calculated MIXMOD means and SDs of components 1 and 2 (3.39 ± 0.67 and 5.80 ± 0.63, respectively) were similar to those determined by dichotomizing at the valley point (3.39 ± 0.67 and 6.02 ± 0.98).

Although platelet 5-HT values were nonnormally distributed in the MR group according to the Anderson- Darling test, this nonnormality was due to four individuals with levels more than 3 SD above the mean. These four apparent outlier individuals were carefully examined but did not differ from the group of MR individuals on any of the demographic, diagnostic, or behavioral measures obtained. When these subjects were excluded, the Anderson-Darling statistic was not significant (A^2_{50} = 0.180, p = .92). In contrast, when the four PDD individuals with 5-HT levels more than 3 SD above the mean were excluded, the Anderson- Darling statistic remained significant (A^2_{77} = 1.03, p = .010). Mixture-modeling analysis in the combined PDD group (n = 77) revealed that a mixture of two normal distributions fit the data better than did a single distribution or a mixture of three distributions. Bayesian information criteria for the one-, two-, and threecomponent distribution models were 131.6, 130.8, and 138.4, respectively. The MIXMOD graphical solution for the preferred two-component model is plotted in Figure 2.2. The MIXMOD calculated means and SDs for the upper and lower components (see figure legend) were similar to those determined above by dichotomization at the valley point.

Relation Between ADI-R-Rated Autistic Behavioral Expression and 5-HT Level

As the first step in an extensive exploratory analysis of possible behavioral associations with platelet 5-HT levels, we examined possible correlations between the 5-HT level, and the ADI-R total score, the social interaction, communication, and stereotyped behavior ADI-R domain scores, and ADI-R subdomain scores in the combined PDD group (autism, Asperger's, and PDD-NOS, n = 81). No significant Spearman correlations were found between any of the ADI-R scores examined and platelet 5-HT levels: r values observed for the total, social interaction, communication, and stereotyped behavior scores and platelet 5-HT level were -0.008, 0.083, -0.064, and 0.044, respectively. A linear regression analysis of the same four behavioral scores also showed no significant association with platelet 5-HT level, with standardized β values ranging from .39 to -.035.

Language Development Items and 5-HT Level

The relationship between several speech and language items from the ADI and platelet 5-HT levels was assessed by comparing 5-HT levels in dichotomized subgroups. There were no significant differences in mean platelet 5-HT levels

between verbal (mean = 4.55 ± 1.50 nmol/10^9 platelets, n = 66) and nonverbal (mean = 5.29 ± 1.72 nmol/10^9 platelets, n = 15; t_{79} = 1.69, p = .096) PDD subjects, and between subjects who spoke their first single word after (4.69 ± 1.47 nmol/10^9 platelets, n = 45) and before (mean = 4.69 ± 1.69 nmol/10^9 platelets, n = 36; t_{79} = 0.01, p = 1.0) the age of 24 months. Mean levels also did not differ in subjects speaking their first sentence after 33 months of age (mean = 4.71 ± 1.61 nmol/10^9 platelets, n = 60) versus those with their first sentence before 33 months of age (mean = 4.61 ± 1.43 nmol/10^9 platelets, n = 21; t_{79} = 0.25, p = .80).

Relations Between Other Behavioral Expression Measures and 5-HT Level

The potential relationships between 5-HT and the total scores and subscores on the CBCL, CY-BOCS, ABC, and CSBQ were examined in the combined PDD group (autism, Asperger's, and PDD-NOS, n = 81) and in the combined developmentally delayed group (PDD and MR, n = 135). No significant correlations were seen in the PDD group; however, in the combined (PDD + MR) group, the ABC total score (r = 0.238, p = 0.006), three of the ABC subscores (Sensory, Body/Object Use, and Sensory, r values 0.253, 0.186, and 0.243, p values .003–.030), two of the CBCL subscales (Thought and Attention Problems, r values 0.172 and 0.173, p = .046), and the CSBQ item Stereotypical/Sensory Problems (r = 0.184, p = 0.033) did tend to correlate with 5-HT level. When these seven scores showing some trend to correlate with 5-HT level were entered into a forward linear regression analysis, only the ABC Sensory score was significantly associated (platelet 5-HT; standardized β = .216, t_{135} = 2.53, p = 0.013). To control for the influence of diagnosis, group status (PDD or MR control) was entered into a linear regression model with ABC Sensory score. This analysis revealed that when controlling for diagnostic group, there was clearly no significant separate effect of severity of PDD-related behaviors as measured with the ABC (group and ABC β weights, .357 and .016, p values <.0001 and .87, respectively).

Behavioral and Demographic Variables in Biochemically Defined Subgroups

To explore any influence of the major behavioral and demographic variables on group membership for hyper- versus normoserotonemia in the PDD group, a logistic regression analysis was performed. In the PDD group (n = 81), none of the variables significantly predicted membership in the hyper- and normoserotonemic groups.

Discussion

Several findings have emerged from this study of large, ethnically homogenous, groups of subjects. As expected, the finding of hyperserotonemia in autism was replicated. The 29% group mean elevation seen in the autistic subjects compared to normal controls was consistent with the study of McBride et al. (1998) and was in the lower end of the range of reported elevations (Anderson et al., 1990; Cook and Leventhal, 1996). Importantly, a significant and substantial elevation of 37% was also seen in the PDD-NOS group. This first report of group mean platelet 5-HT levels in PDD-NOS strongly indicates that platelet hyperserotonemia also occurs in this other category of patients on the autism spectrum. No mean elevation was seen for the Asperger's group, but only five patients were studied. A previous report of platelet 5-HT levels in Asperger's also found no elevation in a small group (n = 5) of subjects (Anderson et al., 1996).

The finding of similar group means and variances for the MR group and the normal control group confirms data reported by McBride et al. (1998) and more firmly establishes the absence of hyperserotonemia in nonautistic MR. The absence of any relationship between intelligence or level of functioning and platelet 5-HT level in either the PDD or MR groups supports the group mean data. Although there are several reports of hyperserotonemia in groups of MR subjects (Schain and Freedman, 1961; Partington et al., 1973; Hanley et al., 1977), the groups studied were small and the subjects were not as well characterized as in the present study. We did note that there were four MR subjects with 5-HT values more than 3 SD above the control mean (> 7.5 nmol/10^9 platelets); however, no distinguishing characteristics could be discerned for these four subjects, and they constituted only 7% of the MR group.

A major finding of the present study was the apparent bimodal distribution of platelet 5-HT levels in the PDD group. While previous studies have consistently found group mean elevations of 5-HT in autism, it has not been possible to draw any conclusions regarding the modality of the measure. Previously, it was not at all clear whether the entire group distribution had shifted upward or whether a hyperserotonemic subgroup existed. The large, homogenous groups that we studied were probably crucial to our being able to detect bimodality. It now appears that approximately half of the PDD subjects closely overlap the normal distribution in

platelet 5-HT level, while a second mode of hyperserotonemic subjects exists. Thus, a biochemically defined subgroup of patients can now be identified and an initial empirically derived cutoff (4.55 nmol/10^9 platelets) now determined for the hyperserotonemia of PDD. Surprisingly, the prevalence of hyperserotonemia was not higher in autism (36%) compared to PDD-NOS (58%).

A range of demographic and clinical variables, including age, pubertal status, gender, use of medication, and season of blood draw, did not appear to influence platelet 5-HT levels. The lack of an age effect is consistent with most previous studies (Ritvo et al., 1971; Anderson et al., 1987; Abramson et al., 1989). The absence of an effect of puberty is at some variance with the report of McBride et al. (1998) that indicated a lowering of platelet 5-HT levels after puberty. Most prior studies had not observed a puberty effect (Ritvo et al., 1971; Anderson et al., 1987; Abramson et al., 1989), and the effect reported by McBride et al. was much less marked in their Caucasian subjects. Two previous studies also suggested that the use of medication (other than reuptake inhibitors) did not substantially influence platelet 5-HT levels (Kuperman et al., 1987; Minderaa et al., 1989). Previously reported seasonal differences have been variable and generally of relatively minor effect (Badcock et al., 1987; McBride et al., 1998).

The absence of any significant behavioral correlates with platelet 5-HT levels or hyperserotonemic status was remarkable given the extensiveness of the behavioral assessments. The language and communication domain was examined in particular depth in this context, given prior reports of possible associations with platelet 5-HT levels (Cook et al., 1990; Cuccaro et al., 1993) and our own limited data indicating that the group mean value of 5-HT was not increased in Asperger's subjects.

In summary, we have replicated the platelet hyperserotonemia of autism in Dutch subjects, found that platelet 5-HT levels are also increased in PDD-NOS, identified bimodality of the measure in autistic and PDD-NOS subjects, and established an absence of a group mean elevation in MR. The absence of detectable behavioral correlates was somewhat surprising, but it will serve to focus our interest on understanding the genetic and biochemical mechanisms underlying the altered platelet 5-HT levels seen in a subgroup of individuals with PDD. The recent report of the extremely high heritability of platelet 5-HT is encouraging in this regard (Ober et al., 2001).

Limitations

Although relatively large, ethnically homogeneous subject groups were studied, sample size still limited the commingling or admixture analysis even in the combined PDD group. Indications of bimodality in the separate autism and PDD-NOS groups are especially tentative. More generally, it is not certain that the findings regarding the distribution of platelet 5-HT levels and the absence of behavioral correlates can be generalized to other populations.

Clinical Implications

If replicated, the defining of normo- and hyperserotonemic subgroups in PDD may provide a route to illuminating the neurobiology and genetics of autism. The biochemically defined groups may also differ in their response to pharmacological and behavioral intervention.

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